IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: SHIN *et al.*
Application No. 10/531,158
Filed: April 12, 2005
Confirmation No. 7490
For: Method for Decreasing Depression by Inhibiting the Activity of N-type Calcium Channel
Examiner: CHERNYSHEV, OLGA N
Art Unit: 1649
Attorney Docket No.: 7037-70886-01

COMMISSIONER FOR PATENTS
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DECALARATION UNDER 37 C.F.R. §1.132

1. We, Hee-Sup Shin and Chanki Kim are co-inventors named in the above-referenced patent application.

2. It is our understanding that claims 1, 4-9 and 11 of the present application are rejected under 35 USC 112, 1st paragraph as lacking enable requirement.

With respect to the above-rejection, we performed supplementary experiments for supporting the enablement requirement. The experimental procedures and result are as follows:

EXPERIMENTAL PROCEDURES

Animals

All animals were handled in accordance with the animal care and use guidelines of the Korea Institute of Science and Technology. Animals were housed in a temperature- and humidity-controlled environment with free access to food and water under a 12-h light/12-h dark cycle. For this study, F1 (C57BL/6J × 129S4/SvJae) male mice (10~15 weeks old) were used for behavioral tests. All behavioral experiments were performed and videotaped by one investigator, and the tape was interpreted by another investigator blinded to the genotypes of the animals used in the experiments.

Local Injection of ω-conotoxin GVIA

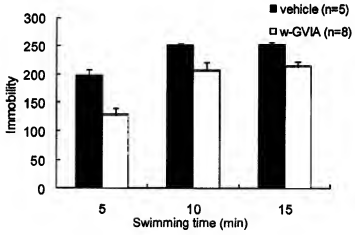
F1 wild-type male mice were anesthetized with avertin, and a guide cannular (Plastics One) for microinjection of an inhibitor α1B subunit of N-type calcium channel , ω-conotoxin GVIA, was stereotaxically placed into the dorsal raphe nucleus (DRN). The stereotaxic coordinates for the micro-infusion were anterior-posterior = – 4.5 mm from the bregma; lateral = ± 1.2 mm; ventral = – 4.0 mm; tilted 22.5 degree. Animals were injected either with vehicle (0.9% NaCl containing 1mg/ml cytochrome C) alone or with ω-conotoxin GVIA (9 ng/0.2 μl/5 min, Alomone) into the DRN 1 hour before the forced swimming test.

RESULT

Phenocopy by Injection of an N-type Ca^{2+} Channel Blocker

To confirm that the decreased depression behaviors of $Ca_v2.2^{-/-}$ mice is due to the defect of N-type Ca^{2+} channel in the DRN, we focally injected an inhibitor α1B subunit of N-type calcium channel, ω-conotoxin GVIA (9 ng/0.2 μl/5 min), into the DRN of wild-type male mice (F1). After 1hr, we performed forced swimming tests. The mice microinjected with ω-conotoxin GVIA showed a significantly reduced immobility in the cylinder compared to the vehicle-treated control mice (Figure). These results indicate that the decreased depression behaviors of $Ca_v2.2^{-/-}$ mice were primarily due to a lack of N-type Ca^{2+} channels in the brain region, such as DRN, and also suggest that a developmental anormaly is not a factor in the expression of the anti-depression phenotype.

FIGURE



Phenocopy experiments in wild-type male mice with ω-conotoxin GVIA microinjected into the DRN (A and B) The drug-injected mice (n = 8) showed a significantly reduced immobility in the forced

swimming test compared to the vehicle-injected control mice (n = 5). * $p<0.05$.

As shown in the figure, ω-conotoxin GVIA which is an inhibitor of α1B subunit of N-type calcium channel can alleviated symptom of depression when administrated into a wild-type mice. Thus, a skilled in the art can acknowledge that the present application complies with 35 USC 112, first paragraph from the above experimental data.

3. We hereby declare that all statements made herein of our own knowledge are true and that all statement made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Shin, Hee-Sup

SHIN, Hee-Sup

July 25, 2008

Date

KIM, Chanki

KIM, Chanki

2008. 7. 25

Date